Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 13973 - 13986
Received 05 March 2021; Accepted 01 April 2021.

Molecular study of some species of the Brassicaceae family

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ABSTRACT

Study complet in postgraduate laboratories at the College of Science, Department of Biology, University of Kufa, with the help of the al'amin Center in the province of Najaf, and a DNA sequence analysis was performed at the macrogen center in the state of South Korea. Five species of the Brassicaceae family have been studied using two different molecular genes: ITS and TRNL-F; Both methods have been used to discover the molecular similarities and differences associated with determining the genetic makeup. Due to DNA amplification and taking its products and sequencing, the results of the analysis of the ITS ribosome and the TRNL-F green plastid gene showed a correlation and the presence of white and red radish in one cover, and the wild radish was located near them, while the watercress and hot duck appeared in another cover at the same distance, But with a genetic distance, which confirms that all species belong to the same family and tribe.

Keywords: Molecular study, Brassicaceae family, DNA

INTRODUCTION

Molecular studies are considered the most important studies to identify the species because they are more accurate and comprehensive in the process of separation and discrimination. And the development of modern scientific devices and life technologies referred to by (Heywood, 2007) and Stace (2005) represented by the invention of the Scanning electron microscope (S.E.M) and the Transmission electron microscope (T.E.M) and rigor in the study of Karyotype has an effective role in the development of studies and solving many complex problems.

In Iraq, Townsend (1980) in Flora if Iraq indicated that there are more than 500 genera and more than 2000 species spread around the world, especially in temperate regions, of which 80 or more are in Iraq, while Al-Musaw (1987) mentioned that this family includes About 380 genera and 3000 species in Iraq have 80 wild genera and 177 species. As for Al-Katib (2000), he mentioned 350 genera and 2,500 species spread over the most in temperate and cold regions in the northern hemisphere, of which 75 are wild and 18 species are cultivated for decorative and food purposes in Iraq. The crucifera family has plants that are distinguished by their great economic importance, as Al-Musawi (1987) mentioned that the plants of this family are widely used as vegetables (such as Raphanus L, Brassica L and Lepidum L) and ornamental plants such as Matthiola L. Also, some species have medicinal importance such as Brassica nigra L.

Among these DNA regions, ITS (or a part of it, ITS2) is one of the most widely used DNA fragments in plant molecular systematics at the generic and species levels because of its potentially high resolution of inter- and intraspecific relationships (Alvarez & Wendel 2003; Buchheim et al. 2011; Staggemeier et al. 2015).

Chloroplast DNA (cpDNA) has many beneficial regions that are not coded in terms of genetics. Due to its evolutionary value, the (trnT - trnF) RNA region has been extensively studied. Intergenic space occurs between the trnL (UAA) 3'exon and the trnF (GAA) gene (Liu et al. 2005). There are two types of exon protected regions in the trnL gene.

Materials and Methods :

Study complet postgraduate laboratories at the College of Science, Department of Biology, University of Kufa, with the help of the al'amin Center in the province of Najaf, and a DNA sequence analysis was performed at the macrogen center in the state of South Korea.

Scientific name	Common name	Tribe
Raphanus sativus Var. *longipinnatus L.*	White radish	**Brassiceae**
Raphanus sativus L. Var .red	Red radish	**Brassiceae**
Raphanus raphanistrum L .	Wild radish or FIHAILA	**Brassiceae**
Eruca sativa Mill.	Rocket or GARGIR	**Brassiceae**
Diplotaxis harra L.	HARA or KHAFAJ	**Brassiceae**

Table (1): Species and varieties included under study from Brassicaceae family with common names and tribes .

Primers:

The primers were diluted with the addition of (ddH2o) water and according to the attached leaflet from the US provider (IDT), the required concentration was prepared by taking 20 µl of the primer solution and completing the volume to 100 µl by adding di-distilled water to make it ready for use. The best ice pack is to take 20 microliters and dissolve it in 80 microliters of double-distilled water and return the box containing the primers to the freezer, which is kept in a refrigerator, and the following table shows the details of each primer :

primer	Sequences (5 → 3')	base

ITS-A	5'-GGAAGGAGAAGTCGTAACAAGG-3	22-MER
ITS-B	5'-CTTTTCCTCCGCTTATTGATATG-3	23 -MER
TRN L	5'-CGAAATCGGTAGACGCTACG-3	20 -MER
TRN F	5'-ATTTGAACTGGTGACACGAG-3	20 -MER

DNA Isolation from Plant Samples:

The Genomic DNA Mini Kit (Geneaid Biotech. Ltd; Taiwan Company) provides a quick and easy method for purifying total DNA (including genomic DNA, mitochondrial and chloroplast DNA) from plant tissue.

Agarose Gel Electrophoresis Analysis:

The gel electrophoresis methods were done according to Sambrook and Russel (2001).

Polymerase chain reaction (pcr) :

The pcr technique was used to amplify the region (ITS 1) and the gene (TrnLF) using primers ITSA, ITSB, TRNL and TRNF provided by the American company (IDT), where the reaction mixture was prepared according to the following steps :

1- The primer was dissolved according to the proportions specified on the primer tube and waited for 45 minutes for the primer to be dissolved.
2- The working solution is supplied from concentrated stock in a ratio of 1: 9 .
3- 2 mL of each forword and reversal gene were taken and placed in a master mix tube (antibody, mediated hot, start tag DNA polymerase , Mgcl2 , Dntps , enhanser and stablizer).

After that, 8 ml of water was taken and the volume was completed to 20 ml by adding 10 ml of water RNAse_fre.

After that, the samples were entered into the Thermal Cycler (PCR) device according to special conditions for each primer

Result and discasion

Phylogenic analysis relies on one of the most important semantics used by researchers to clarify the historical and evolutionary relationships between neighborhoods. In recent times, there has been a prominent increase in research that has focused on finding evolutionary relationships in cruciferous plants, including the species of the Brassiceae clan, and for the purpose of obtaining the genetic tree that clarifies the relations of convergence and divergence between Plants and to complete the analysis the gene and region must be determined, so the ITS ribosomal region and the TRNL-F chloroplast region were selected. Knowledge of the sequence is a useful tool for resolving ambiguities and overlaps and finding evolutionary relationships between related species and to complete the sequence. Some analysis software has been used, including (UPGAM, Maximum Likelihood, Neibor Join) to study the relationships between some types of the Crusader family that were put under study.

4-2-1 DNA extraction :

DNA was extracted from five leaves of different species belonging to different races of the family Brassicaceae Which were collected during the duration of the study only and the results of the electrical relay showed the efficiency of the method Extraction using the Genomic DNA Mini Kit, plate (1).

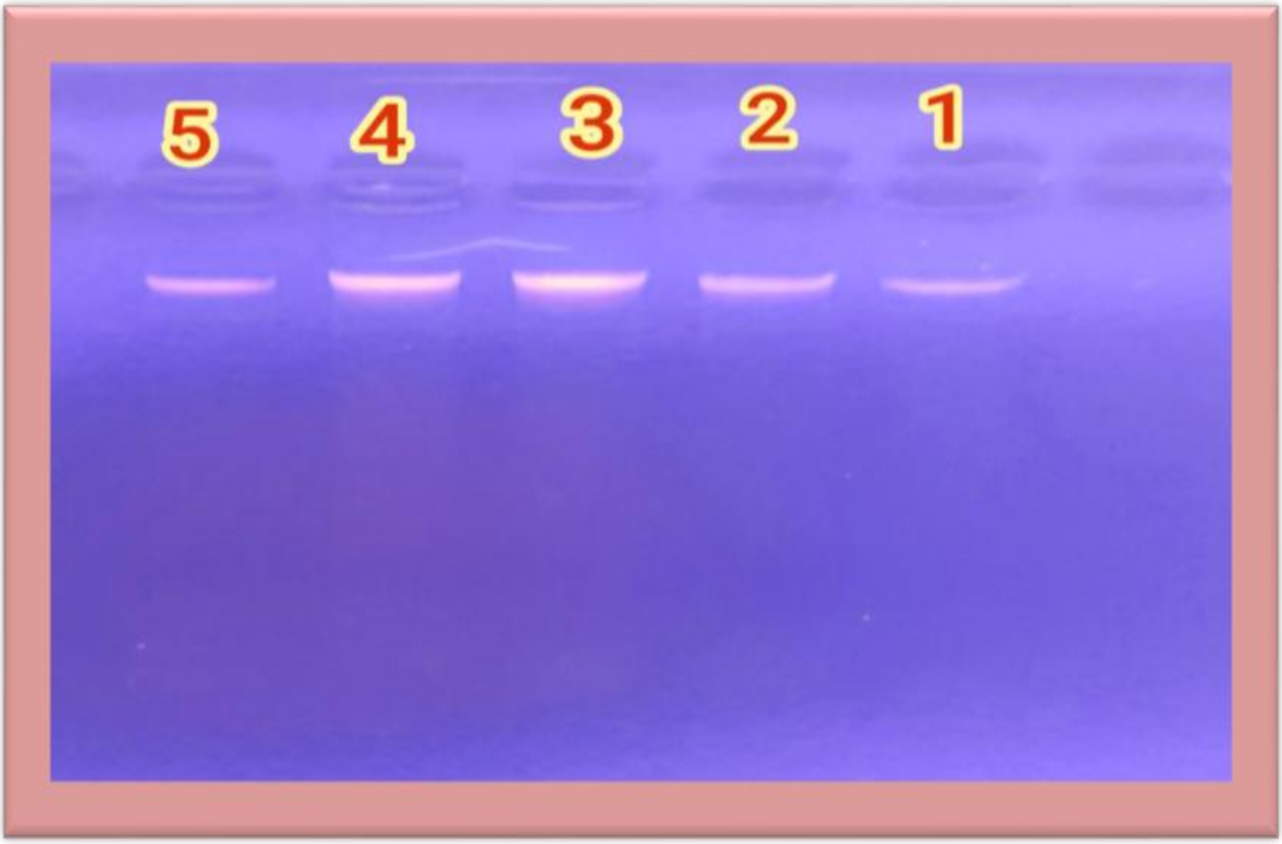

Plate (1)The transfer of DNA extracted from the leaves of some species: (*1-Raphanus sativus* Var *longipinnatus* L. 2-*Raphanus raphanistrum* L . *3-Raphanus sativus* L. *Var.* 4- *Eruca sativa* Mill. 5-*Diplotaxis harra (Forssek)*,Bossi).

4-2-2 PCR Amplification of ITS region :

Results showed amplification of DNA extracted from or thinner of some species and genera of the spring family Using the initial pair ITS A, ITS B
To amplify the ITS spacer the nitrogen base sequence of the target region
The results of the amplification showed a polymerase product of 500 - 600 base pairs in length For all species, with a similar genotype as in plate(2).

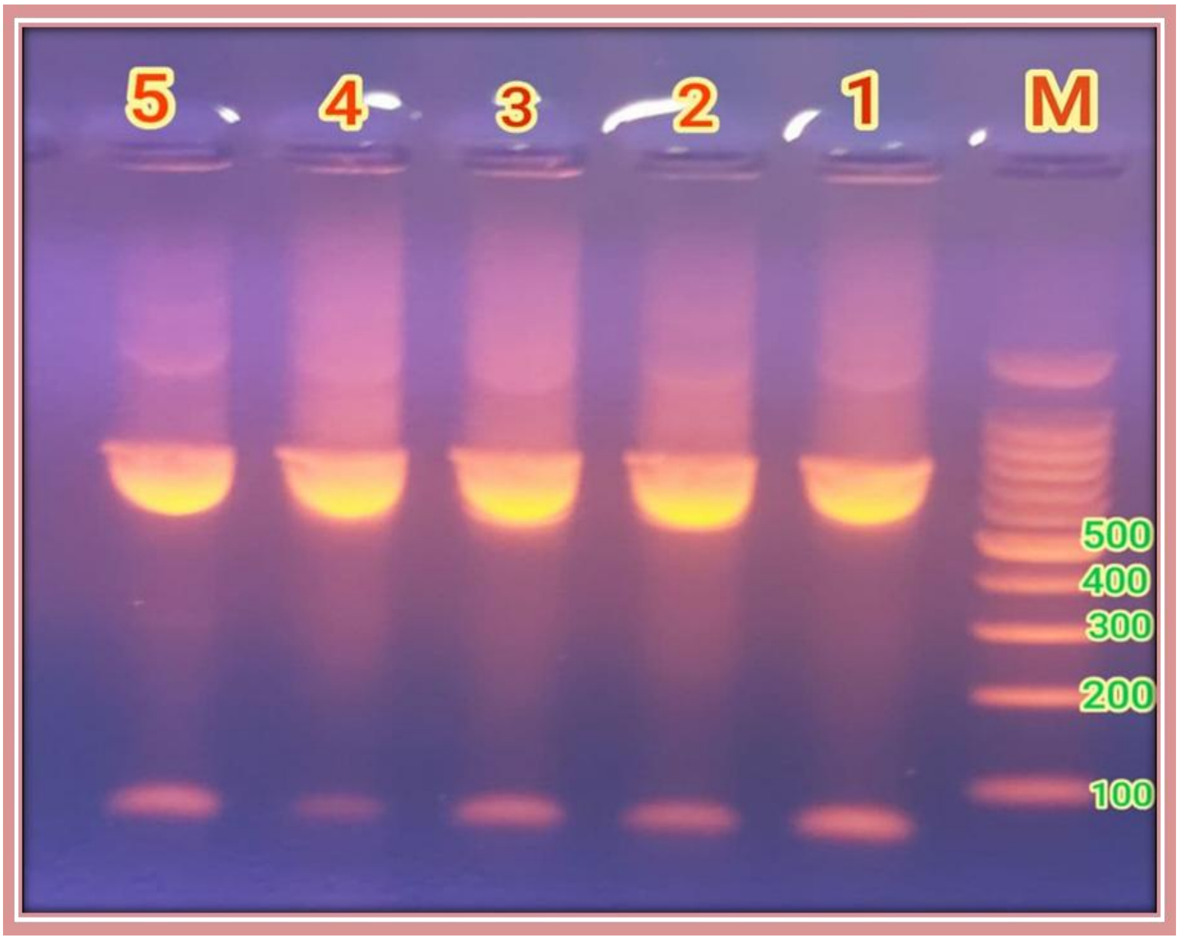

Plate (2) Polymerization products using the ITS starter pair of (5) species of cruciferae family.

M - Refers to the molecular parameter with a weight of 100 base pairs - (base pair = bp).

1- *Raphanus sativus longipinnatus L.*
2- *Raphanus raphanistrum L.*
3- *Raphanus sativus L. Var.*
4- *Eruca sativa Mill.*
5- *Diplotaxis harra (Forssek),*

4-2-3 PCR Amplification of TRNL-F region :

Results showed amplification of DNA extracted from or thinner of some species and genera of the spring family Using the initial pair TRNL, TRNF

To amplify the TRNL-F the nitrogen base sequence of the target region

The results of the amplification showed a polymerase product of 500 – 600 base pairs in length For all species, with a similar genotype as in plate(3).

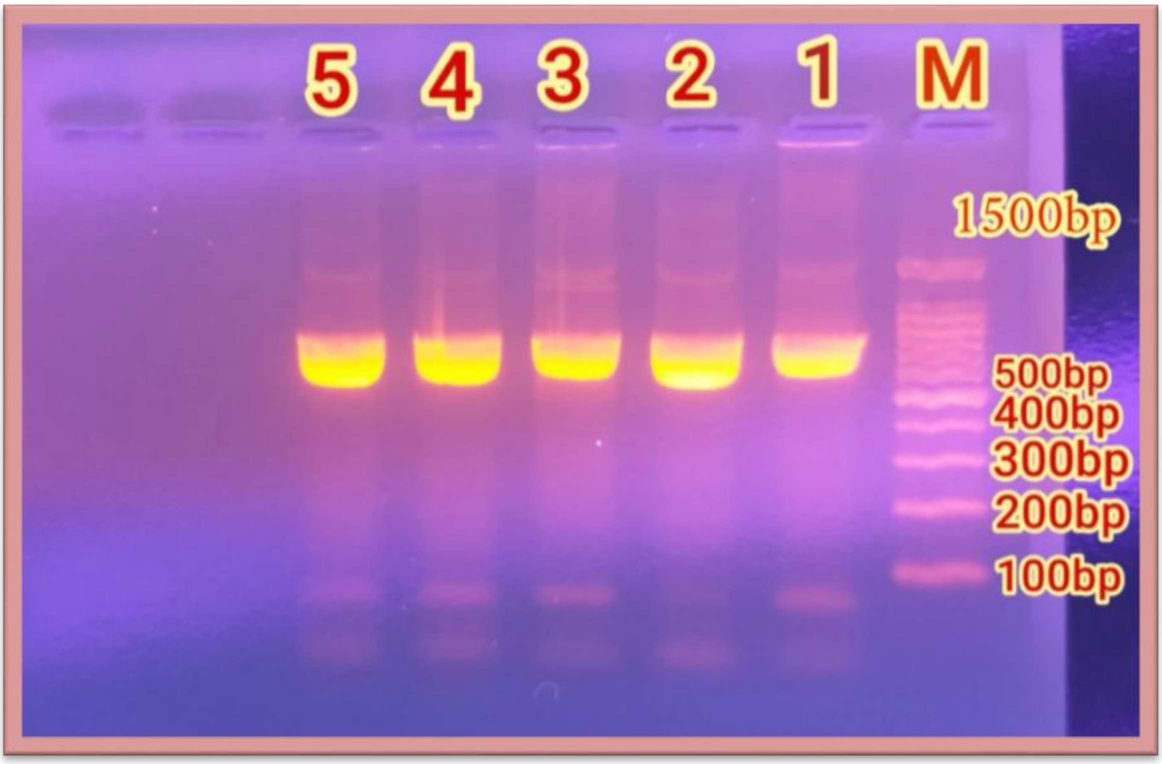

Plate (3). Polymerization products using the TRNL-F starter pair of (5) species of cruciferae family.

M - Refers to the molecular parameter with a weight of 100 base pairs – (base pair = bp).

1- *Raphanus sativus* Var . *longipinnatus L.*
2- *Raphanus raphanistrum* L.
3- *Raphanus sativus* L.
4- *Eruca sativa Mill.*
5- *Diplotaxis harra* , (Forssk.) Boiss.

ITS region sequence analysis in the studied species :

The sequence was studied in the ITS ribosomal region, and a highly supportive genetic tree was obtained and the analysis was conducted with the three aforementioned software, respectively. From the results of the analysis in the UPGAM program, a genetic tree was obtained consisting of two main clades, the first containing the two species *Eruca sayiva* and the type *Diplotaxis harra*, with a degree of reliability of the data. The entry amounted to 100% and is considered a sister of the second clad, and the degree of reliability of the data entered reached 100%, as well as the second clad, in turn, comprising two secondary cladding, the first containing the two species *Raphanus sativus Longipinatus L*. and *Raphanus sativus L.Var* , Their data was 100% reliable, and the other secondary clad was isolated, *Raphanus raphanistrum.* Figure (1).

It was not much different when analyzing with a cap program such as aMaximumlikelihood, as the tree was closely distinguished from the previous tree, closely related species were grouped together, but the support for data reliability was relatively less than the tree produced by the UPGMA analysis as the Raphanus species was distinguished.Raphanus sativus Longipinatus L and reached reliability 86% with Raphanus . The coatings of Eruca sayiva and Diplotaxis harra were , while support decreased to 72% in Raphanus raphanistrum clades. Figure (2).

When analyzing the sequence data using the NeiborJoin program, we find that the support was better than the previous program, as the clade of the Raphanus sativus Longipinatus L. was distinguished by a degree of reliability and reached 93% with the type Raphanus sativus L. Var. The second clad was divided into secondary cladids, as the clad of the two species Eruca was distinguished sayiva and Diplotaxis harra with more support than its predecessor, as the data reliability reached 87%, while the support reached 71% in the clad of Raphanus raphanistrum. Figure (3).

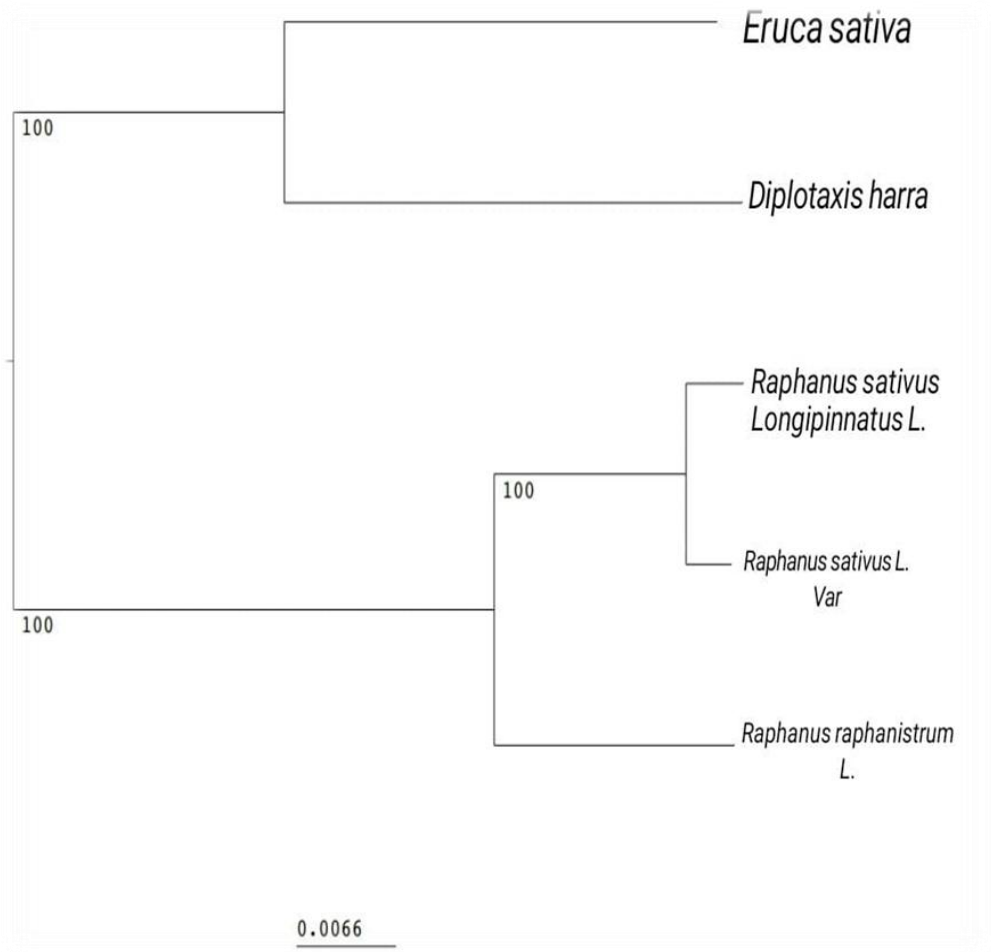

Figure (1) Genetic tree of the studied species in the ITS region using UPGAM analysis software.

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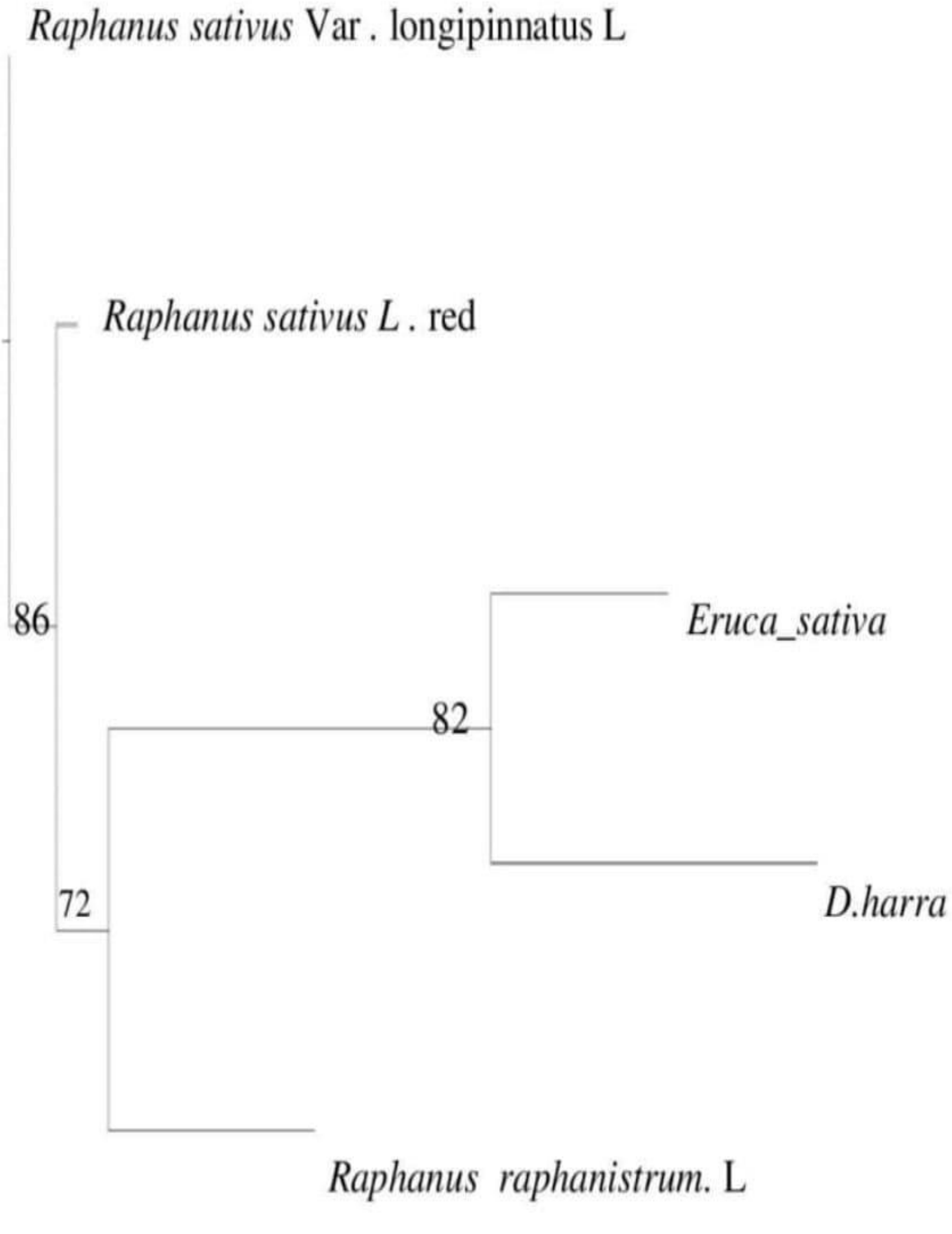

Figure (2) Genetic tree of the studied species in the ITS region using Maximum liklihood analysis software.

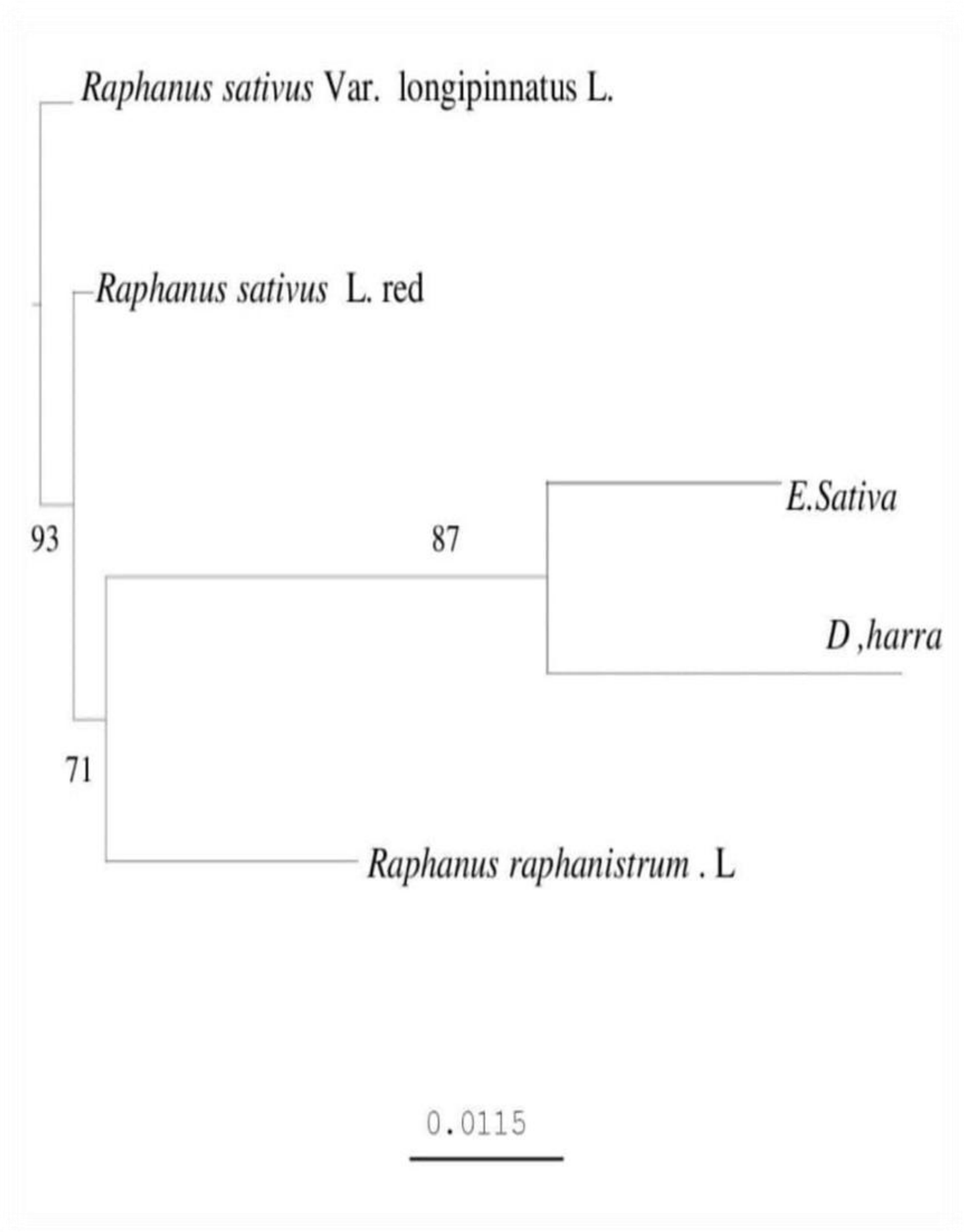

Figure (3) Genetic tree of the studied species in the ITS region using Neibor Join analysis software.

4-2-5 TRNL-F region sequence analysis in the studied species :

If we follow the TRNL-F gene sequence for the five types studied by the three programs that were mentioned previously, we find that the tree is the same in all programs. In the UPGMA program, we find that the tree was divided into two main cladding, the first includes cladin for the species *Eruca sativa and Diplotaxis harra*, with high data support reaching 96% As for the other secondary clad, which included the two species *Raphanus sativus Longipinatus L. and Raphanus sativus L. Var*, with high data support, it reached 100%, and within the second clad, the species *Raphanus raphanistrum* came in a single clad and was isolated in it. Figure (4) **.**

The situation was not different when performing the analysis using the Maximum likeilihood program, as the resulting tree did not differ in shape, but the support for data reliability was different In the clad, which includes the two types *Eruca sativa and Diplotaxis harra*, the reliability of the data was 78% . *Raphanus sativus Longipinatus L. and Raphanus sativus* L. Var. We find the reliability in it is excellent, reaching 96%, while the single clad of *Raphanus raphanistrum*, which is considered a sister to the other clad, remained. Figure (5).

The same is when performing the analysis using the Neibor join program, as the resulting tree did not differ in the shape and distribution of the species within the clades. In the first and second major cladids, the support between them reached 53%, while in the secondary clad, which represents the two species *Raphanus sativus Longipinatus L. and Raphanus sativus L.* Var. The reliability was distinguished, reaching 100%, while the single clag was still the share of *Raphanus raphanistrum*, which is considered a sister of the second clad. (6).

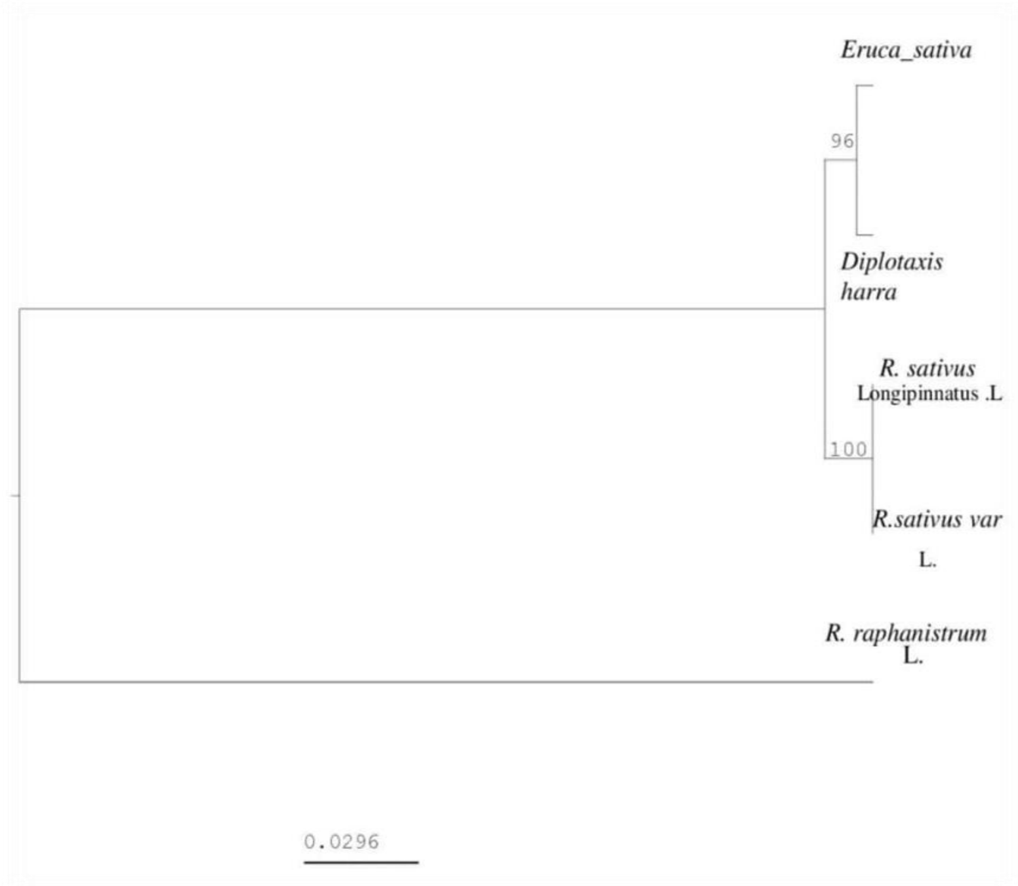

Figure (4) Genetic tree of the studied species in the TRNL-F region using UPGAM analysis software.

Figure (5) Genetic tree of the studied species in the TRNL-F region using Maximum liklihood analysis software.

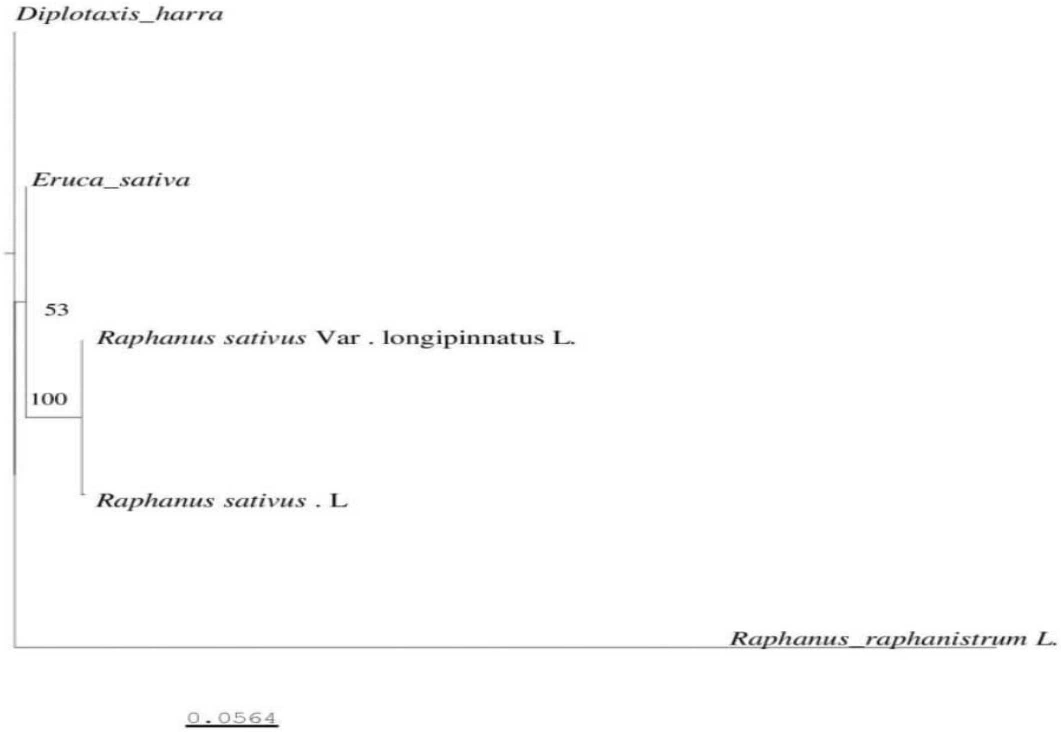

Figure (6) Genetic tree of the studied species in theTRNL-F region using Neibor Join analysis software.

The great advances in DNA sequencing techniques have reflected positively on the abundance of DNA sequencing data and thus lead to finding a latent source for the molecular information used in building the phylogenetic tree of evolutionary relationships between plants between plants. Researchers face many challenges or difficulties in finding efficient and effective testing strategies in In the current study, the (ITS) ribosomal region and the (TRNL_F) gene were chosen to know the sequence of 5 types of the Crucifera family. The (ITS) region and the (TRNL_F) gene were widely used to solve the overlap between plant groups and to give a view of the evolutionary evolutionary relationships between and within the species. Overlapping, including Crucifera families, was in agreement with Bleeker et al. (2002), Koch et al. (2003), and Ali et al. (2010), who used the ITS region and the TRNL_F gene to find phylogenetic relationships between the family plants under study.

As for the sequence analysis and drawing of the genetic tree of the studied species, there are several methods of constructing the genetic tree, including what is used in the current study of the UPGMA program, the Maximum Liklihood program, the Neibor Join program, and other excellent programs that were not used in this research, such as the Maximum Parsimony program and the program. Bayesian analysis This agrees positively with what was used and confirmed by Cialdella et al. (2010), Crawley & Hilu (2012), Hamasha et al., (2012) and Burton et al., (2004) and this is due to the software's efficiency in analyzing the sequence and rim tree Genetic convergence and divergence between species and genus beings.

The pcr technique is the adopted basis for molecular classification and thus the sequence knowledge of plant species depends mainly on the purity of the DNA extracted from the samples. This is what the current study mechanism achieved by obtaining good polymerization products and thus the sequencing mechanism for most of the studied species, the (ITS) region, and the (TRNL_F) gene. This was confirmed by Baldwin *et al.,* (1995).

It was found from the results of the current study and through the completion of the process of amplifying and purifying DNA using the primer ITS and TRNL_F, thus obtaining knowledge of the sequence and analysis that it is the only raphanistrum of the genus Raphanus is unique to an independent clade alone, as is the case for Raphanus sativus longipinntus L. and Raphanus sativus L. that came. Within one clad Also, Eruca sativa and Diplotaxis harra came to another clad alone and is considered the sister of the other clad , the one was isolated from the other with good data reliability for the ITS region and the TRNL_F gene, and with the three analyzes, the results revealed a close relationship between the species because they descended from one family and one clan, in agreement with that , Al-Shehbaz *et al .,* (2006) and Chen *et al* ., (2015). And Warwick *et al* ., (2010).

It is worth noting that this is the first study on the level of Iraq that deals with the study of some types of the Crusader family at the genetic level of the ITS ribosomal region, and the TRNL-F gene, as there was a single study by Al-Edahari (2015), for the Poaceae family.

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